

# FIG. 1A

## Nucleotide sequence of EOS (SEQ.ID.NO1)

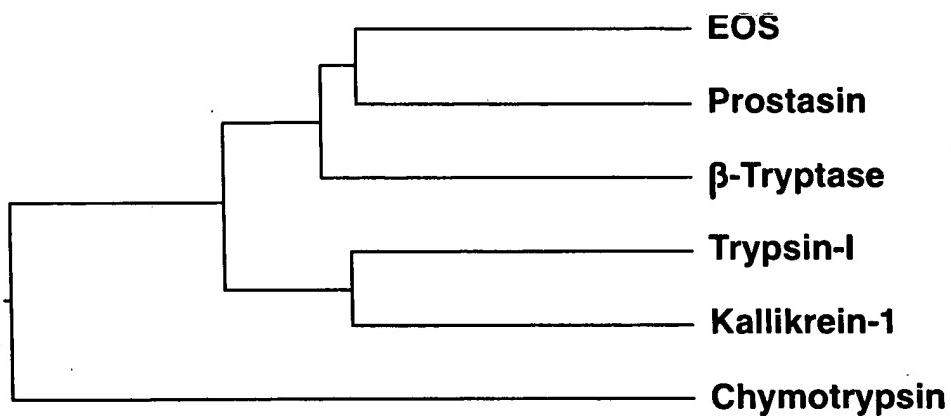
CCACGCGTCCGACCAGAGTCCAAGCCCTAGGCAGTGCCACCCCTAACCAAGCCCAGCCTTG  
 AAGACAGAATGAGAGGGGTTCCCTGTCTCCAGGTCTGCTCCTCTGGTGCTGGAGCTG  
 CTGGGACTCAGGGAAAGGAAGTCTGCAGCCTGCCGGCAGCCCCGATGTCCAGTCGGATCG  
 TTGGGGGCCGGATGGCCGGACGGAGAGTGGCCGTGGCAGGCGAGCATCCAGCATCCTG  
 GGGCACACGTGTGCGGGGGTGCCTCATGCCCGGAGTGGGTGCTGACAGCGGCGACT  
 GCTTCCCCAGGAGGGCACTGCCAGCTGAGTACCGCGTGCCTGGGGCGTGCCTGCGTCTGG  
 GCTCCACCTCGCCCCGACGCTCTGGTGCCGTGCGACGGTGCTGCTGCCCGGACT  
 ACTCCGAGGACGGGGCCCGCGGACCTGGCACTGCTGCAGCTGCGTGCCTGGGGCGTGCCTG  
 TGAGCGCTCGCGTCCAACCGTCTGCCCTGCCGTGCCGGCGCCGCCGCCGCC  
 CACCATGCCGGTCACCGGCTGGGCAGCCTCCGCCAGGAGTGCCCTCCCAGAGTGGC  
 GACCGCTACAAGGAGTAAGGGTGCCGCTGGACTCGCGCACCTGCCACGGCCTCTACC  
 ACGTGGCGCGGACGTGCCCGAGGCTGAGCGCATTGTGCTGCCCTGGAGTCTGTGCC  
 GCTACCCCCAGGCCACAAGGACGCCCTGCCAGGGTATTCTGGGGACCTCTGACCTGCC  
 TGCAGTCTGGAGCTGGCTCTGGTGGCGTGGTAGCTGGGCAAGGGTTGTGCCCTGC  
 CCAACCGTCCAGGGTCTACACCAGTGTGCCACATATAGCCCCTGGATTCAAGCTCGC  
 TCACCTCTAATGCTAGCCGGTGAGGCTGACCTGGAGCCAGCTGCTGGGTCCCTCAGCCT  
 CCTGGTCATCCAGGCACCTGCCATACCCACATCCCTCTGCCCTGGAGGCCAAGATGC  
 CTAAAAAAGCTAAAGGCCACCCACCCCCACCCACCCACCTCTGGCTCCTCTCCTCTT  
 GGGGATCACCAAGCTCTGACTCCACCAACCCATCCAGGAATCTGCCATGAGTCCCAGGG  
 AGTCACACTCCCCACTCCCTCTGGCTTGATTTACTTTCTGGCCCTGGCCAGGGCT  
 GGGCGCAAGGCACGCAGTGATGGCAAACCAATTGCTGCCATCTGGCTGTGTGCCCAT  
 CTTTTCTGGAGAAAGTCAGATTACAGCATGACAGAGATTGACACCAGGGAGATCCTC  
 CATAGCTGGCTTGAGGACACGGGACACAGCCATGAGCGGCCCTCTAAGAGCTGAGAGA  
 CAGCCGGCAGGGAAATCGGAACCCCTCAGACCCACAGCCGAAGGCAGTGGATTCTGGCAGC  
 ACCCTGAAGGAGCTGGGAAGTAAGTTCTCCCCAGCCTCCAGATAAGAGCCCCGCCGGCC  
 AATCCCTCATTTCAACCTAAAGAGACCCCTAAGCAGAGAACCTAGCTGAGCCACTCCTGA  
 CCTACAAAAGTTGTGACTTAATAATGTGTGCTTAAGCTGCCAAAAAAAAAAAAA

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 100-4209-4400

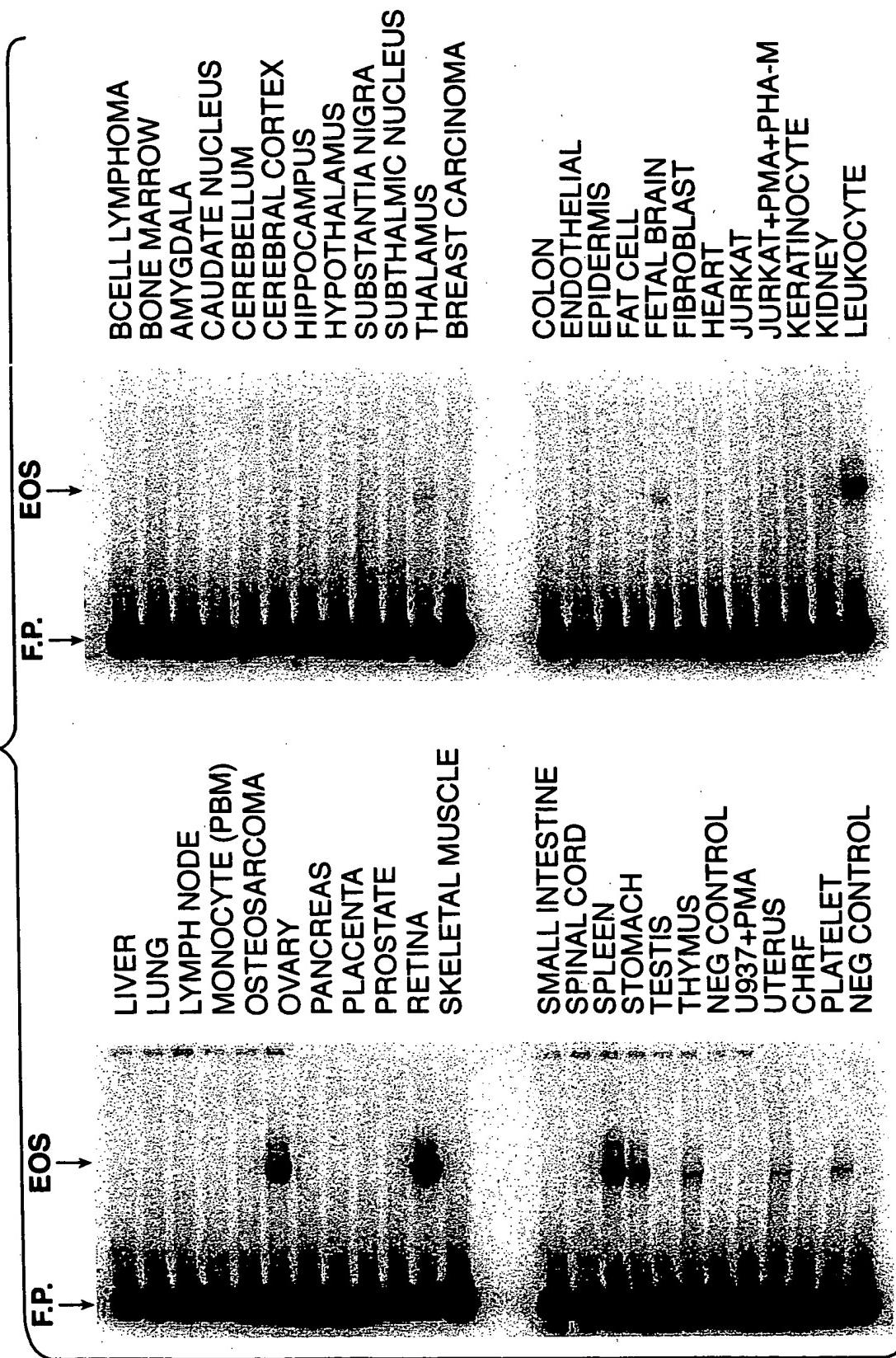
**FIG. 1B****Amino Acid sequence of EOS protease (SEQ.ID.NO.:7)**

Met Arg Gly Val Ser Cys Leu Gln Val Leu Leu Leu  
 Leu Val Leu Gly Ala Ala Gly Thr Gln Gly Arg Lys  
 Ser Ala Ala Cys Gly Gln Pro Arg Met Ser Ser Arg  
 Ile Val Gly Gly Arg Asp Gly Arg Asp Gly Glu Trp  
 Pro Trp Gln Ala Ser Ile Gln His Pro Gly Ala His  
 Val Cys Gly Gly Ser Leu Ile Ala Pro Gln Trp Val  
 Leu Thr Ala Ala His Cys Phe Pro Arg Arg Ala Leu  
 Pro Ala Glu Tyr Arg Val Arg Leu Gly Ala Leu Arg  
 Leu Gly Ser Thr Ser Pro Arg Thr Leu Ser Val Pro  
 Val Arg Arg Val Leu Leu Pro Pro Asp Tyr Ser Glu  
 Asp Gly Ala Arg Gly Asp Leu Ala Leu Leu Gln Leu  
 Arg Arg Pro Val Pro Leu Ser Ala Arg Val Gln Pro  
 Val Cys Leu Pro Val Pro Gly Ala Arg Pro Pro Pro  
 Gly Thr Pro Cys Arg Val Thr Gly Trp Gly Ser Leu  
 Arg Pro Gly Val Pro Leu Pro Glu Trp Arg Pro Leu  
 Gln Gly Val Arg Val Pro Leu Leu Asp Ser Arg Thr  
 Cys Asp Gly Leu Tyr His Val Gly Ala Asp Val Pro  
 Gln Ala Glu Arg Ile Val Leu Pro Gly Ser Leu Cys  
 Ala Gly Tyr Pro Gln Gly His Lys Asp Ala Cys Gln  
 Gly Asp Ser Gly Gly Pro Leu Thr Cys Leu Gln Ser  
 Gly Ser Trp Val Leu Val Gly Val Val Ser Trp Gly  
 Lys Gly Cys Ala Leu Pro Asn Arg Pro Gly Val Tyr  
 Thr Ser Val Ala Thr Tyr Ser Pro Trp Ile Gln Ala  
 Arg Val Thr Ser Asn Ala Ser Arg

## **FIG. 2**



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**FIG. 3**

**FIG. 4A**Eco RI

GAATTCAACCACCATGGACAGCAAAGGTTCGTCGCAGAAATCCCGCCTGCT  
 1 -----+-----+-----+-----+-----+-----+-----+-----+ 50  
 CTTAAGTGGTGGTACCTGTCGTTCCAAGCAGCGTCTTAGGGCGGACGA  
 M D S K G S S Q K S R L L  
 Prolactin Signal Sequence

CCTGCTGCTGGTGGTGTCAAATCTACTCTTGTGCCAGGGTGTGGTCTCCG  
 51 -----+-----+-----+-----+-----+-----+-----+ 100  
 GGACGACGACCAACACAGTTAGATGAGAACACGGTCCCACACCAGAGGC  
 L L L V V S N L L L C Q G V V S  
 Prolactin Signal Sequence

Not I

ACTACAAGGACGACGACGACGTGGACGCCGCGCTTGCTGCCCTTT  
 101 -----+-----+-----+-----+-----+-----+-----+ 150  
 TGATGTTCCCTGCTGCTGCTGCACCTGCGCCGGCGAGAACGACGGGGAAA  
 D Y K D D D D V D A A A L A A P F  
 FLAG EK Pro

Xba I

GATGATGATGACAAGATCGTTGGGGCTATGCTCTAGAGGACGGAGAGTG  
 151 -----+-----+-----+-----+-----+-----+-----+ 200  
 CTACTACTACTGTTCTAGCAACCCCCGATACGAGATCTCCTGCCTCTCAC  
 D D D D K I V G G Y A L E D G E W  
 EK Pro

**FIG. 4B**

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201     GCCGTGGCAGGCGAGCATCCAGCATCCTGGGGCACACGTGTGCAGGGGGT  
 250     -----+-----+-----+-----+-----+  
       CGGCACCGTCCGCTCGTAGGTCGTAGGACCCCCGTGTGCACACGCCCCCA  
       P W Q A S I Q H P G A H V C G G  
 \_\_\_\_\_ Protease EOS.CDS \_\_\_\_\_

251     CGCTCATCGCCCCCCCAGTGGGTGCTGACAGCGGCGCACTGCTTCCCCAGG  
 300     -----+-----+-----+-----+-----+  
       GCGAGTAGCGGGGGGTACCCACGACTGTCGCCGCGTGACGAAGGGGTCC  
       S L I A P Q W V L T A A H C F P R  
 \_\_\_\_\_ Protease EOS.CDS \_\_\_\_\_

301     AGGGCACTGCCAGCTGAGTACCGCGTGCCTGGGGCGCTCGTCTGGG  
 350     -----+-----+-----+-----+-----+  
       TCCCGTGACGGTCGACTCATGGCGCACGCCGCGACGCAGACCC  
       R A L P A E Y R V R L G A L R L G  
 \_\_\_\_\_ Protease EOS.CDS \_\_\_\_\_

351     CTCCACCTCGCCCCGCACGCTCTCGGTGCCGTGCGACGGGTGCTGCTGC  
 400     -----+-----+-----+-----+-----+  
       GAGGTGGAGCGGGCGTGCGAGAGCCACGGGCACGCTGCCACGACGACG  
       S T S P R T L S V P V R R V L L  
 \_\_\_\_\_ Protease EOS.CDS \_\_\_\_\_

**FIG. 4C**

401 CCCCGGACTACTCCGAGGACGGGGCCCGCGCGACCTGGCACTGCTGCAG  
 +-----+-----+-----+-----+-----+-----+-----+  
 GGGGCCTGATGAGGCTCCTGCCCGGGCGCCGCTGGACCCTGACGACGTC  
 P P D Y S E D G A R G D L A L L Q  
 \_\_\_\_\_ Protease EOS.CDS \_\_\_\_\_

451 CTGCGTCGCCCGGTGCCCTGAGCGCTCGCGTCCAACCCGTCTGCCCTGCC  
 +-----+-----+-----+-----+-----+-----+-----+-----+  
 GACGCAGCGGGCCACGGGGACTCGCGAGCGCAGGTTGGGCAGACGGACGG  
 L R R P V P L S A R V Q P V C L P  
 \_\_\_\_\_ Protease EOS.CDS \_\_\_\_\_

501 CGTGCCCCGGCGCCCGCCGCCGGCACACCATGCCGGGTACCCGGCT  
 +-----+-----+-----+-----+-----+-----+-----+  
 GCACGGGCCGCGGGCGGGCGGCGGGCCGTGTGGTACGGCCCAGTGGCCGA  
 V P G A R P P P G T P C R V T G  
 \_\_\_\_\_ Protease EOS.CDS \_\_\_\_\_

551 GGGCAGCCTCCGCCAGGAGTGCCCTCCAGAGTGGCGACCGCTACAA  
 +-----+-----+-----+-----+-----+-----+-----+  
 CCCCGTCGGAGGCGGGTCCTCACGGGGAGGGTCTCACCGCTGGCGATGTT  
 W G S L R P G V P L P E W R P L Q  
 \_\_\_\_\_ Protease EOS.CDS \_\_\_\_\_

**FIG. 4D**

GGAGTAAGGGTGCCGCTGCTGGACTCGCGCACCTGCGACGGCCTTACCA  
 601 CCTCATTCCCACGGCGACGACCTGAGCGCGTGGACGCTGCCGGAGATGGT  
 G V R V P L L D S R T C D G L Y H

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Protease EOS.CDS

CGTGGGCGCGGACGTGCCCAAGGCTGAGCGCATTGTGCTGCCCTGGGAGTC  
 651 GCACCCCGCGCCTGCACGGGGTCCGACTCGCGTAACACGACGGACCCTCAG  
 V G A D V P Q A E R I V L P G S

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Protease EOS.CDS

TGTGTGCCGGCTACCCCCAGGGCCACAAGGACGCCCTGCCAGGGTGATTCT  
 701 ACACACGGCCGATGGGGTCCGGTGTTCCTGCGGACGGTCCCACTAAGA  
 L C A G Y P Q G H K D A C Q G D S

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Protease EOS.CDS

GGGGGACCTCTGACCTGCCCTGCAGTCTGGGAGCTGGGTCCCTGGTGGCGT  
 751 CCCCCTGGAGACTGGACGGACGTCAGACCCCTCGACCCAGGACCACCCGCA  
 G G P L T C L Q S G S W V L V G V

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Protease EOS.CDS

**FIG. 4E**

Protease EOS.CDS

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Protease EOS.CDS

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Xba I

---

Not I

---

+ 850

+ 900

+ 950

+ 1000

801 GGTGAGCTGGGGCAAGGGTTGTGCCCTGCCAACCGTCCAGGGGTCTACA  
 -----+-----+-----+-----+-----+-----+-----+  
 CCACTCGACCCCGTTCCAACACGGGACGGGTTGGCAGGTCCCCAGATGT  
 V S W G K G C A L P N R P G V Y

851 CCAGTGTGCCACATATAGCCCCTGGATTCAAGGCTCGCGTCACTTCTAAT  
 -----+-----+-----+-----+-----+  
 GGTCACACCGGTGTATATCGGGGACCTAAGTCCGAGCGCAGTGAAGATTA  
 T S V A T Y S P W I Q A R V T S N

901 GCTTCTAGATAACCCTACGATGTGCCCGATTACGCCGCTAGACATCACCA  
 -----+-----+-----+-----+-----+  
 CGAAGATCTATGGGGATGCTACACGGGCTAATGCGGCGATCTGTAGTGGT  
 A S R Y P Y D V P D Y A A R H H H

951 TCACCATCACTAGCGGCCGCTTCCCTTAGTGAGGGTTAATGCTTCGAGC  
 -----+-----+-----+-----+-----+  
 AGTGGTAGTGATGCCGGCGAAGGGAAATCACTCCCAATTACGAAGCTCG  
 H H H \*

HA/HIS-TAG

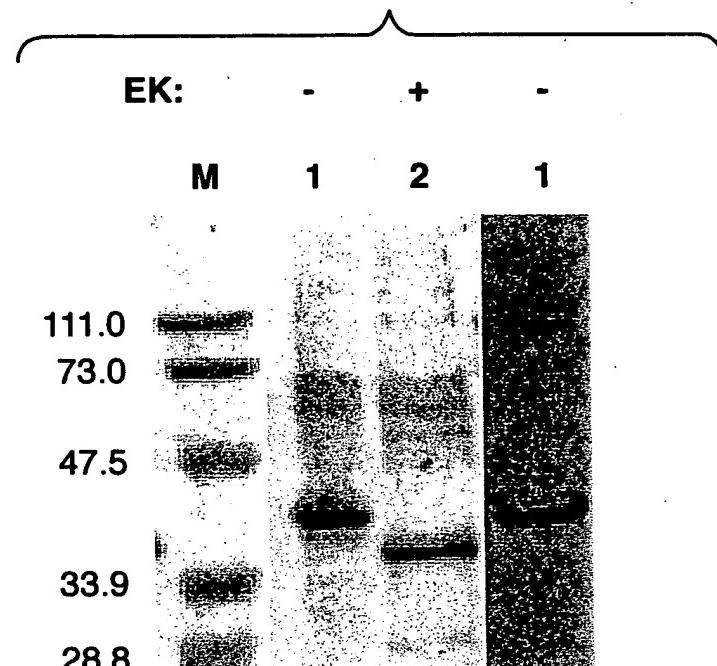
HA/HIS-TAG

10042091-D1602

**FIG. 4F**

SV40 Late pA

1001	AGACATGATAAGATACACATTGATGAGTTGGACAAACCACAACTAGAATGC -----+-----+-----+-----+-----+	1050
	TCTGTACTATTCTATGTAACCTACTCAAACCTGTTGGTGTGATCTTACG	
<hr/>		
1051	AGTGAAAAAAATGCTTATTGTGAAATTGTGATGCTATTGCTTATT -----+-----+-----+-----+-----+	1100
	TCACTTTTTACGAAATAAACACTTAAACACTACGATAACGAAATAAA	
<hr/>		
SV40 Late pA		
1101	GTAACCATTATAAGCTGCAATAAACAGTT -----+-----+-----+-----+	1130
	CATTGGTAATATTGACGTTATTGTTCAA	
<hr/>		

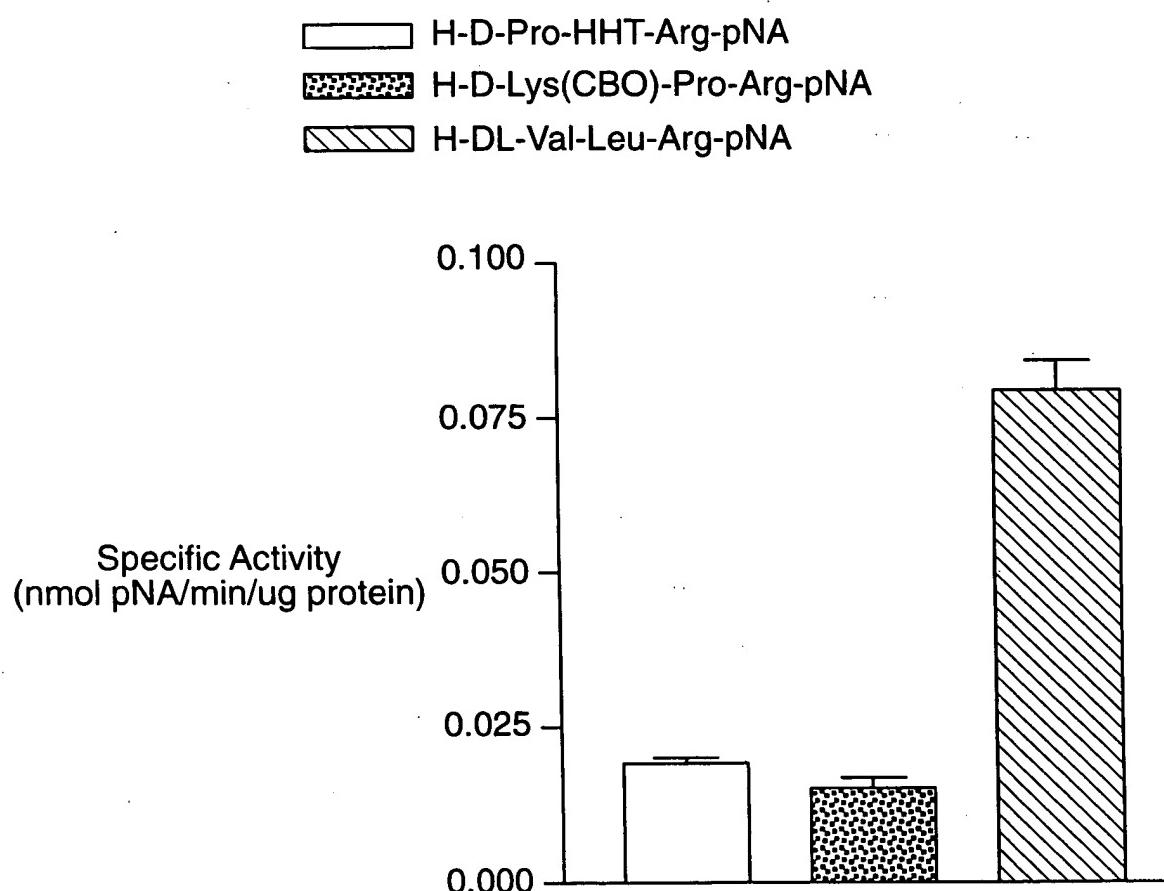
**FIG. 5**

Protease: PFEK2-EOS-6XHIS

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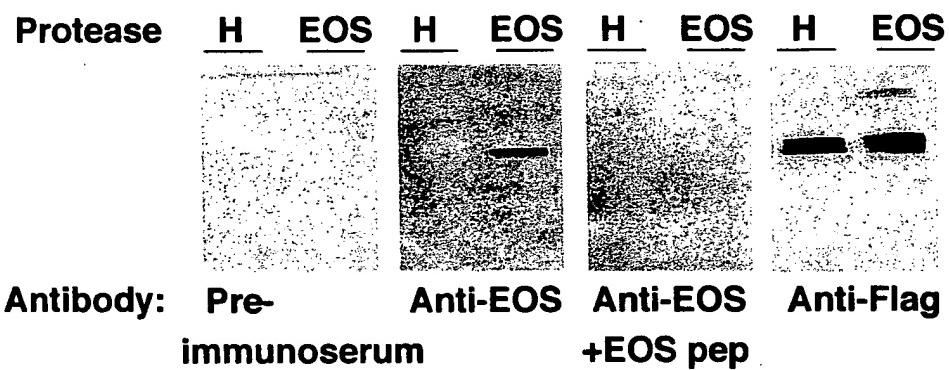
**FIG. 6**

## **Protease Activity of EOS**



**FIG. 7**

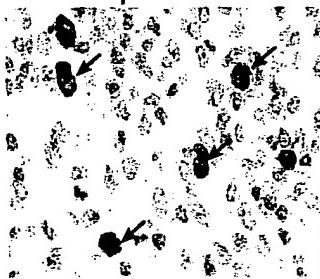
## Anti-EOS Antiserum Immunoblot Characterization



## FIG. 8

Localization of EOS protein (top) and mRNA (bottom)  
in human spleen, lung, and colon

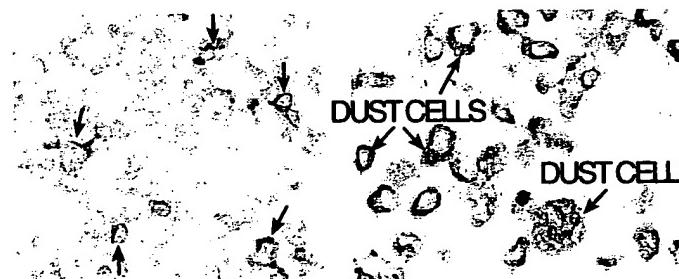
Spleen:



Lung:



Colon:



## FIG. 9

Double immunofluorescence (IF:IF) of  
EOS and macrophage marker:CD68



EOS in human colon

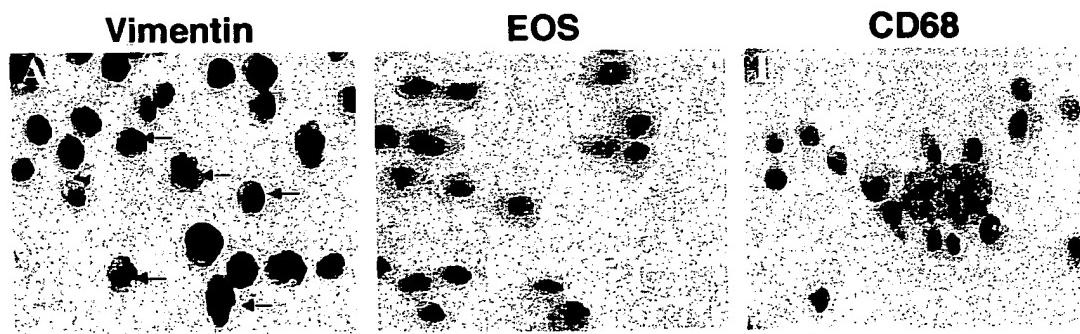


CD68 in human colon

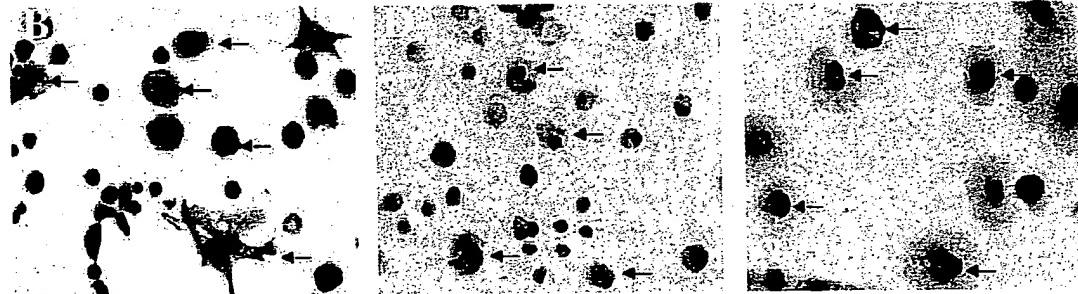
## FIG. 10

Up-regulation of EOS protein by PMA in U937 cells

Untreated U937 cells:



PMA treated U937 cells:



## FIG. 11

Up-regulation of EOS mRNA by PMA in U937 cells

